CATION REGULATION AND SURVIVAL OF THE RED ALGA, PORPHYRA PERFORATA, IN DILUTED AND CONCENTRATED SEA WATER[1]

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Scott and Hayward (1953, 1954), Biebl (1956), and one of us (Eppley, 1958a, 1958b) have considered that a significant feature of ion transport in algae may lie in maintaining a relatively constant intracellular ionic environment, *i.e.*, cellular homeostasis. That transport mechanisms are of survival value is attested to by the great salinity tolerances established for intertidal algae (Biebl, 1952, 1953, 1956, 1958). Although there is increasing interest in homeostatic mechanisms in marine organisms (*e.g.*, Bullock, 1958), data on ion regulation in algae during osmotic stress are scant.

Blinks (1951) has reported the accumulation of K by *Valonia* from slowly concentrating sea water. *Ulva lactuca* loses some K in 70 per cent sea water which is reaccumulated when external Na is returned to normal (Scott and Hayward, 1955).

Ion transport has been implicated in a number of algae in addition to those mentioned. Bergquist (1958a) has studied Na and K movements after desiccation in *Homosira banksii,* MacRobbie and Dainty measured K and Na fluxes in *Rhodymenia palmata* (MacRobbie and Dainty, 1958a) and in the brackish water *Nitellopsis* (MacRobbie and Dainty, 1958b). In each case a high cytoplasmic K content and an Na content somewhat lower than that of the medium are to be noted.

To a degree, salinity tolerances of algae correlate with their intertidal zonation (Biebl, 1952). However, differences are less marked among intertidal and deeper water forms. Obviously other factors are also involved in intertidal zonation and Doty (1946) contends that the duration of exposure, *i.e.*, submergence or emergence, is of prime importance.

Doty and Archer (1950) further suggest that bright sun, rain, and freezing weather are, in that order, the most critical factors in intertidal algal survival. Kanwisher (1957) has recently reported the effects of freezing and drying on the respiration of some intertidal algae. The present paper reports a study of cellular cation concentrations in *Porphyra perforata,* a red intertidal alga, in different sea water concentrations and the role of ion transport in response to osmotic stress is discussed.

Methods

Tissue volumes. Tissue water was taken as the difference between fresh weight (after blotting twice with tissue paper) and dry weight (24 hours at

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105° C.), divided by the fresh weight. Apparent free space (AFS) (Briggs and Robertson, 1957) was obtained by allowing $S^{35}O_4$ to diffuse into the algal tissues from sea water for two minutes, blotting twice, then placing the tissues in 10 ml. distilled water for 10 minutes. Aliquots of the distilled water containing the labelled sulfate were then counted with gas flow apparatus. The results are expressed as volume per unit fresh weight. Apparent osmotic volume (AOV) (Briggs and Robertson, 1957), corresponding roughly to the microscopically estimated cytoplasmic volume, was taken as the difference between tissue water and AFS for each sea water concentration.

Ion contents. Sodium and potassium contents were determined by flame photometry. Results are expressed in units milli-equivalents per kilogram fresh weight (meq./Kg. FW), or milli-equivalents per liter AOV.

Artificial sea waters. One hundred per cent sea water was prepared according to the following formula: NaCl, 0.55 *M*; $MgSO_4$, 0.027 *M*; $MgCl_2$, 0.027 *M*; $CaCl_2$, 0.01 *M*; KCl, 0.01 *M*. The concentrations were adjusted proportionately for diluted and concentrated sea waters. In some cases diluted sea water was prepared by adding distilled water to natural sea water. Calcium chloride was omitted for Ca-free sea water.

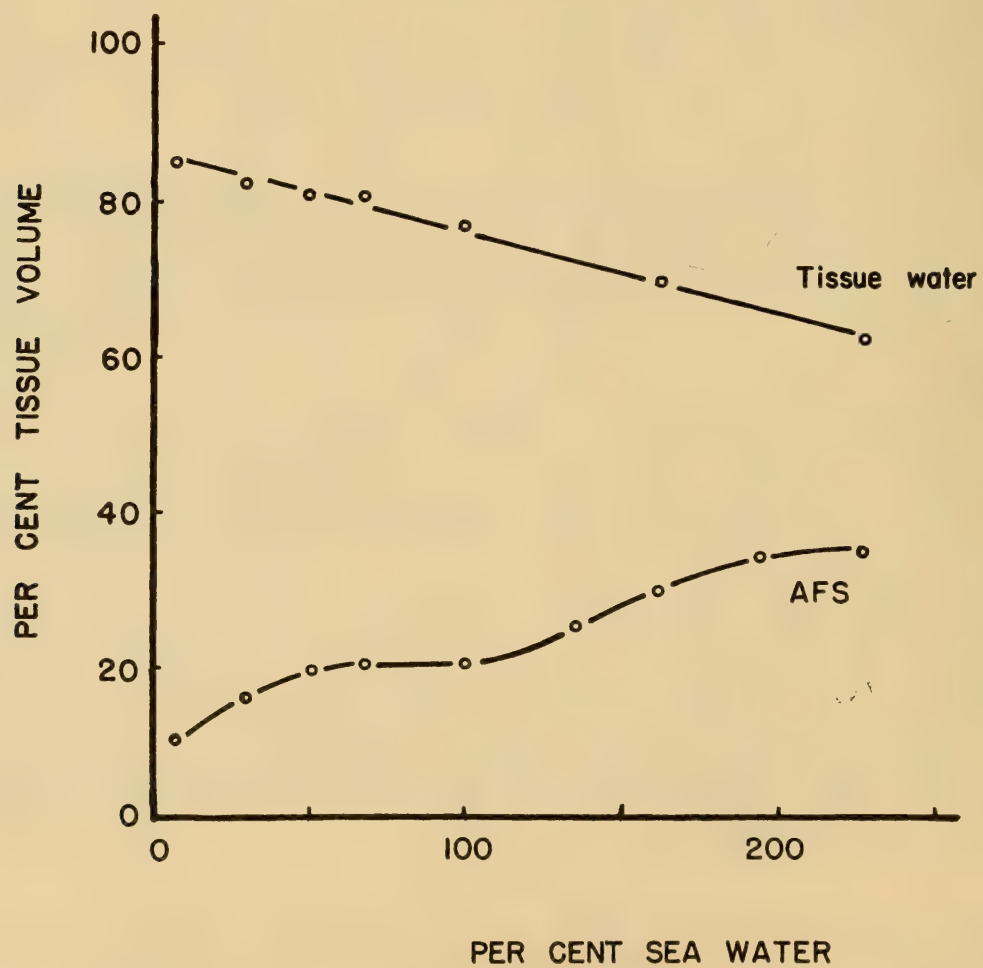

FIGURE 1. Tissue water and apparent free space (AFS) of *Porphyra perforata* as functions of sea water concentration. Apparent osmotic volume is taken as the difference between the two curves.

Results

Tissue volumes at different sea water concentrations. Tissue water and AFS are shown as functions of sea water concentration in Figure 1. AOV is taken as the difference between the two curves. Individual points refer to samples taken

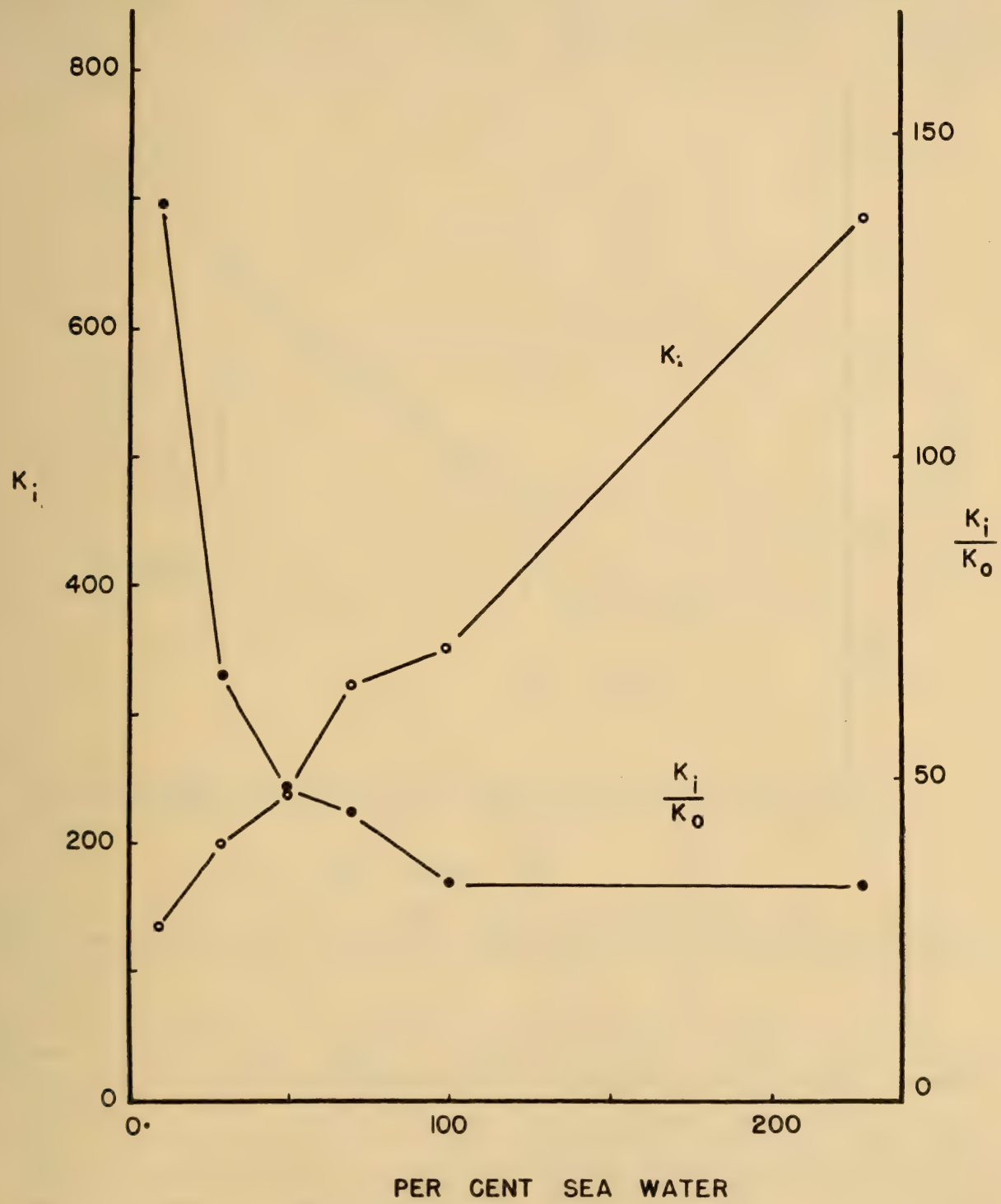

Figure 2. Potassium content (K_i), expressed on the basis of apparent osmotic volume (meq./L. AOV), and potassium accumulation ratios (K_iK_o) for *Porphyra perforata* after 24 hours in different sea water concentrations.

after 24-hour exposure. However, samples taken at two minutes give identical results. Volume equilibration takes place very rapidly in the monostromatic algal tissues.

Tissue water follows a linear relationship with concentration. Dry weight provides an increasing component of tissue weight with increasing concentration. Values for weight changes at 50 and 150 per cent sea water are similar to those reported for shrinking and swelling of *Porphyra tenera* blades (Ogata and Takada, 1955).

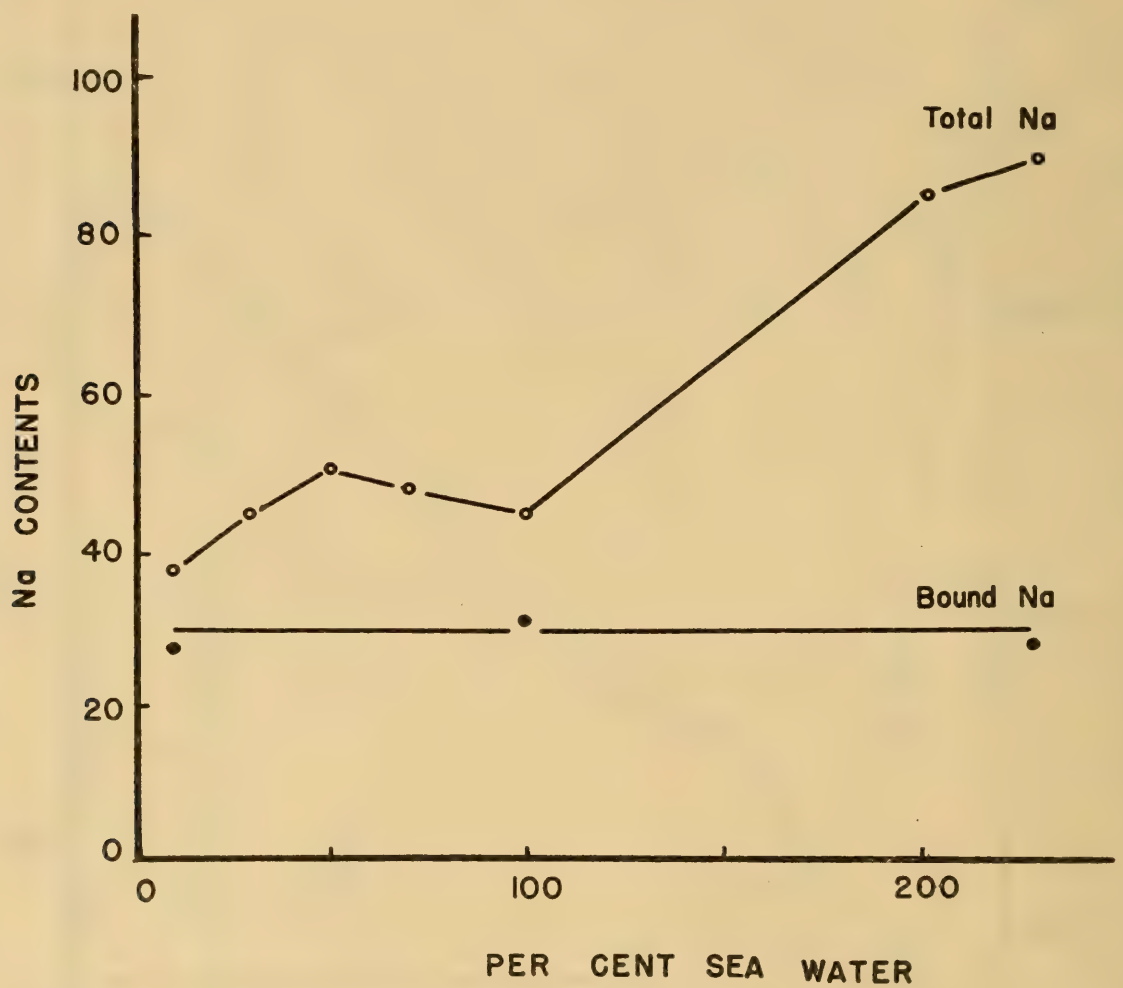

FIGURE 3. Total tissue sodium and bound sodium (the latter determined for killed tissues) in *Porphyra perforata* after 24 hours at different sea water concentrations. Units meq./Kg. FW.

Decrease in AFS in diluted sea water suggests that swelling of the cells squeezes water from the intercellular spaces. At high concentrations the reverse may hold, the extracellular material shrinking disproportionately from the cells. That the intercellular material, a galactan sulfate (Eppley, 1957), is not free to swell indefinitely is indicated by Miwa's study of *Porphyra tenera* (Miwa, 1940). In this species, and probably also in *P. perforata,* at least three structural carbohydrates exist. "Outer membranes," forming a sandwich about the blades, probably restrict swelling. Miwa found these polysaccharide membranes to be hydrolized only with difficulty. The galactan sulfate lies between and immediately

surrounds the cells, each of which has its own discrete wall. None of the three polysaccharides could be identified by Miwa as cellulose.

AOV, taken as an estimate of cytoplasmic volume, shows a relationship to concentration which is reverse to that followed by AFS. Swelling is noted in diluted sea water, a fairly constant volume is maintained between 50 to 100 per cent sea water, and above this value the volume decreases. The cells do not behave as perfect osmometers, nor do they show typical plasmolysis. Both these characteristics are likely due to the extracellular polysaccharides, each of which seems to have distinct swelling and shrinking characteristics. We have no evidence

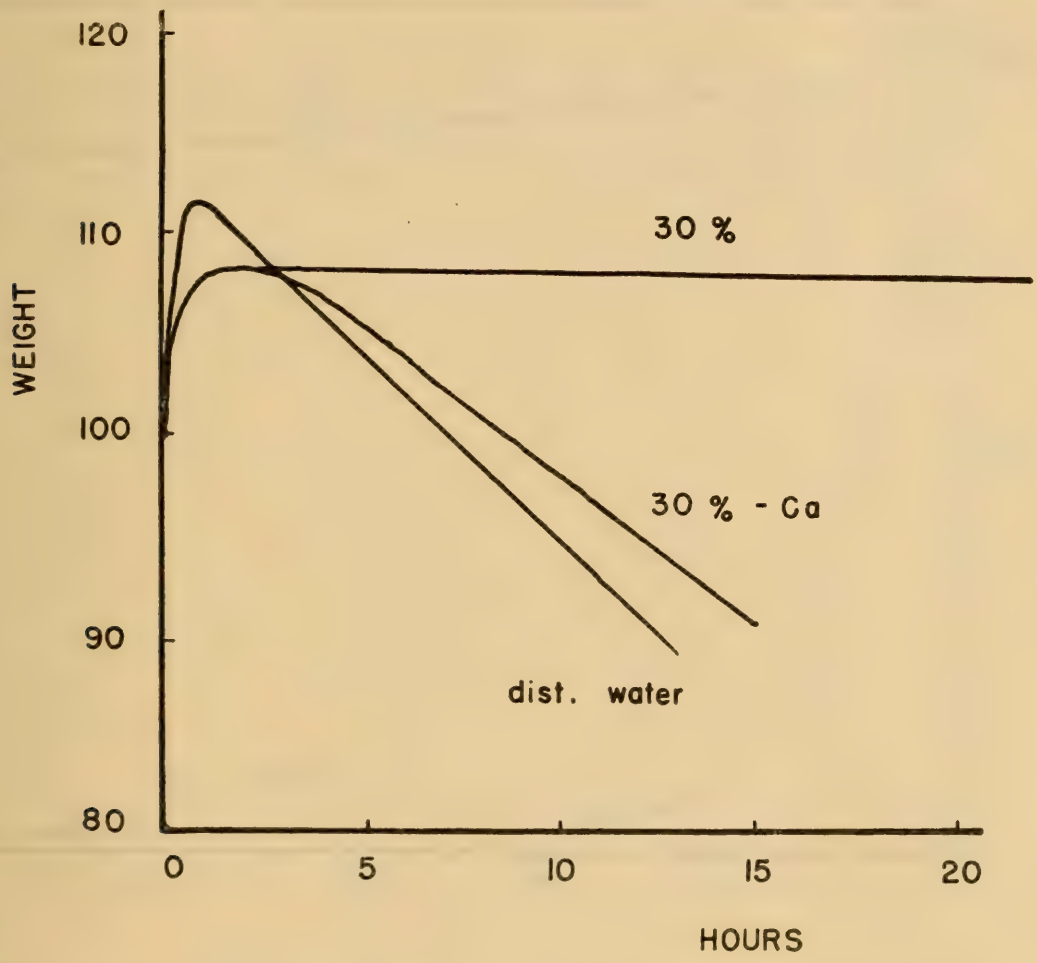

FIGURE 4. Weight, expressed as per cent of the initial weight, of *Porphyra perforata* tissues in 30 per cent sea water with Ca, 30 per cent sea without Ca, and in distilled water. Time course.

that the volume control between 50 and 100 per cent sea water is due to water secretion. Nor does there seem to be any necessity for proposing this as an explanation.

Ion contents in different sea water concentrations. K contents, expressed on an AOV basis, and K_i/K_o accumulation ratios are shown in Figure 2. Cell K follows a linear function of sea water concentration, although a 35-fold, or greater, accumulation is evident throughout. Considerable retention of K occurs in diluted sea waters, resulting in high accumulation ratios, although the actual K content

is lower here than in 100–200 per cent sea water. Little K is bound by the extracellular polysaccharides (1–2 meq./Kg. FW), although large amounts of K may be adsorbed to structural components in other algae, such as *Homosira* (Bergquist, 1958a). Potassium is the principal cellular cation of *Porphyra* at all concentrations studied.

The distribution of Na is less clear, due to appreciable extracellular adsorption. Amounts bound by killed tissues are independent of concentration of sea water for

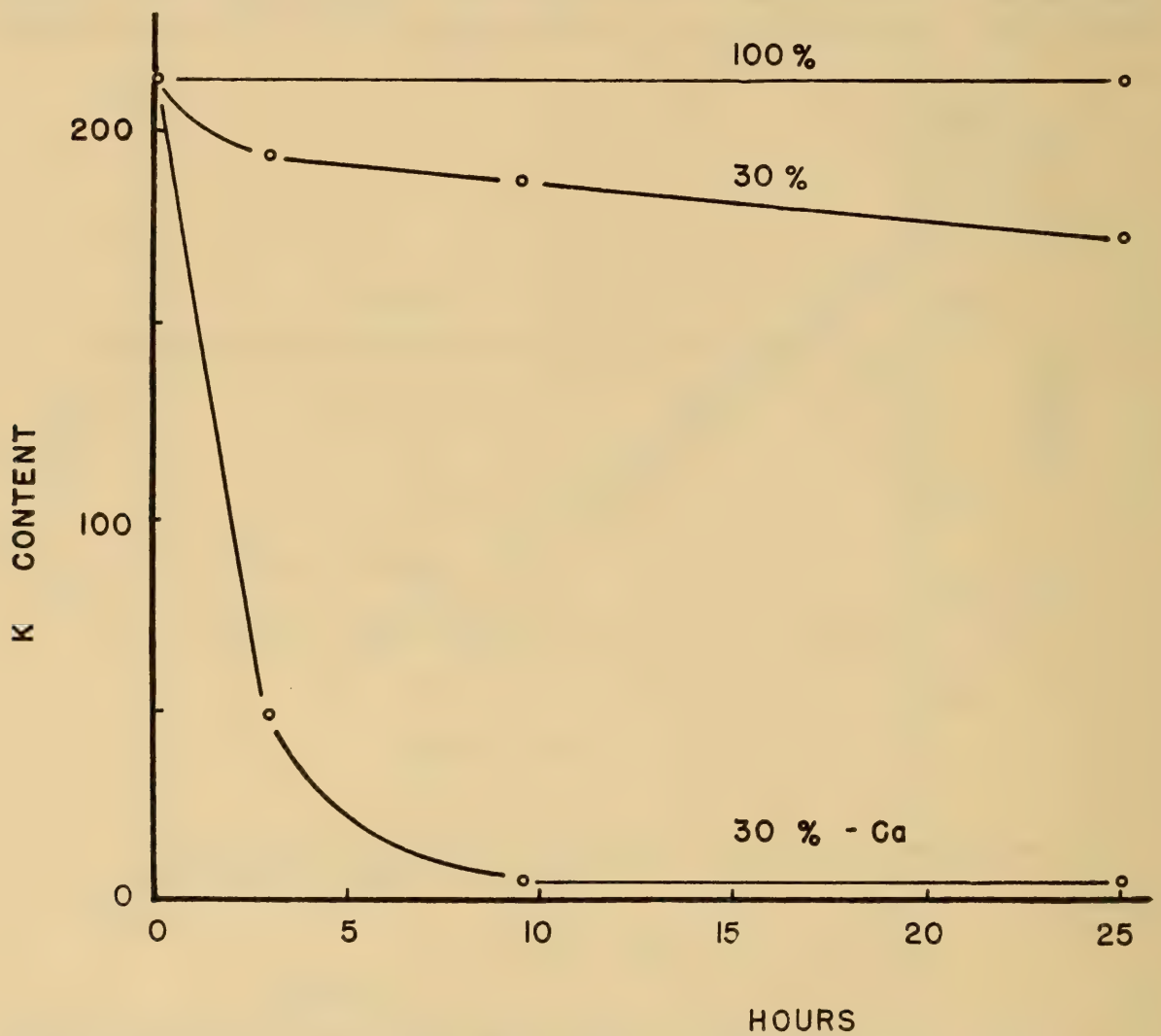

FIGURE 5. Potassium contents (meq./Kg. FW) of *Porphyra perforata* tissues in 100 per cent sea water, 30 per cent sea water with Ca, and 30 per cent sea water without Ca. Time course.

a given plant (Fig. 3), but considerable variation in bound Na is evident in different plants. Therefore Na contents are not expressed on an AOV basis. Throughout the concentration range studied, however, Na is excluded by the cells. The degree of exclusion varies with concentration, but is approximately 10-fold at 100 per cent sea water and increases with increasing sea water concentration.

Total K plus Na thus varies in a roughly linear fashion with salinity, while K is accumulated and Na partially excluded throughout. A similar situation is re-

ported for several halophilic bacteria (Christian and Ingram, 1959). In these bacteria the freezing point depression of cell sap varies directly with sea water concentration, with K contributing significantly to cell osmotic pressure, as does Na.

Calcium and survival in diluted sea water. Tissues placed in 30 per cent sea water without calcium, or in distilled water (Fig. 4) lose weight rapidly after an initial increase. Addition of calcium chloride, 0.01 *M*, prevents the weight loss.

Calcium-free 30 per cent sea water also results in rapid loss of K (Fig. 5), while with Ca the K loss is much depressed. Cell Na, data not shown, increases slightly without Ca (with the concentration gradient), but remains at a lower and constant value with Ca.

TABLE I

Potassium accumulation and sodium extrusion in the presence and absence of $CaCl_2$ or $SrCl_2$ (10 mM/L.). Tissues were first agitated in K-free sea water 20 to 30 hours to render them low in K and high in Na so that subsequent net transport could be measured. They were then transferred to sea water plus KCl.

Expt.	Duration of expt. in hours	K uptake meq./Kg. FW	Na extrusion meq./Kg. FW
1	1.5		
initial		0	0
−Ca		59	26
+Ca		55	38
2	1.5		
initial		0	0
−Ca		46	41
+Ca		60	57
3	19.5		
initial		0	0
−Ca		−7	13
+Ca		96	47
4	16.5		
initial		0	0
−Ca		15	13
+Ca		50	31
+Sr		57	33

Calcium in net transport of K and Na. To investigate the role of Ca in net uptake of K and extrusion of Na, tissues were rendered low in K and high in Na by soaking them in K-free sea water (Eppley, 1958b). They were then placed in sea water with KCl (10 or 20 meq./L.) and the ion contents of the tissues were followed in time. Initial uptake of K and extrusion of Na (Table I) is independent of Ca presence. However, after longer periods net active movements are reduced, indicating loss of K and gain of Na in the absence of Ca. Strontium ($SrCl_2$, 0.01 *M*) may substitute for Ca in preventing loss of K and gain of Na.

Our results are best explained by assuming that Ca-lack gradually brings about leakage through the cell membranes, resulting in increasing movements of salts along their concentration gradients. It seems unlikely that Ca plays any direct

role in the operation of ion transport mechanism, *i.e.,* active transport, but it could well be required to maintain K:Na selective sites on the membrane, or participate in labile membrane structure.

Discussion

Tissue volumes. Because of the lack of a central vacuole and because microscopic estimates of AFS correspond roughly to those measured with $S^{35}O_4$, we have assumed that AFS in *Porphyra* includes the extracellular water but no cytoplasmic component, as is suggested for some higher plant tissues (Briggs and Robertson, 1957). With these assumptions any tissue water not corresponding to AFS must be cellular. AFS has been measured with sucrose for tissues in 100 per cent and diluted sea waters, with results identical to those obtained with radiosulfate. Whether the experimental values include all the extracellular water may be open to question, as well as whether a cytoplasmic component is included. Interestingly, Bergquist (1958b) reported that KCN increases AFS values in *Homosira.* Whether this represents an actual increase in AFS or modification of anionic binding sites was left an open question.

It is with the above reservations that we have taken AOV as the difference between tissue water and AFS, and have calculated K concentrations on this basis.

We do not necessarily presume a uniform cytoplasmic distribution of K, although we plan to investigate this point further. Some localization may occur in vacuomes which are variable in number, but which may comprise a small fraction of cytoplasmic volume. These are visible with neutral red staining. Mitochondria, the chromatophores, and local membrane vesiculations, if they occur, could be sites of variation in cytoplasmic ion concentrations.

While *Porphyra* cells do not behave as perfect osmometers, our results are consistent with the view that active water movements do not take place. Variations in AOV with salinity seem to be due to different degrees of shrinking and swelling of the protoplast and the extracellular, structural polysaccharides.

Ion regulation in concentrated sea water. In corroboration of Biebl's findings (Biebl, 1953), we find that *Porphyra* may survive for some time in 200 per cent sea water. Over the studied range, external ion concentrations of the medium increase equally. Selective precipitation of salts, *i.e.,* $CaSO_4$, occurs only with concentration above 300 per cent. Our investigations have not extended to such concentrations.

Accumulation ratios for K are identical for 100 and 200 per cent sea water. Cell Na shows some increase with concentration, but is lower than that of the medium; thus cation selectivity is retained.

In these experiments changes in salinity were abrupt. Coenocytic algae such as *Valonia* (Blinks, 1951) do not tolerate such rapid changes. Lack of a large central vacuole in *Porphyra* cells may be of importance in this regard. There is no plasmolysis and thus no mechanical injury to the cell membranes with rapid salinity change. AFS and AOV adjustments are almost immediate, as indicated by identical AFS values at two minutes and 24 hours of exposure to concentrated sea water.

Porphyra perforata grows between the 3- and 3.5-foot tide levels, referred to San Francisco (Doty, 1946). Here that alga is regularly covered and uncovered

by the tide twice daily. The maximum period of exposure is 6–8 hours. Concentration of sea water between the algal blades during this period seems unlikely to exceed that tolerated in our experiments, even on the hottest days of summer. Thus we feel that osmotic stress under these conditions is insufficient to result in breakdown of ion transport, resulting in death. Survival in concentrated sea water encountered in the field is not a problem, in our view.

A more serious problem to the emerged algae on hot days is heating, and possibly photoxidation. In summer the superficial algal blades are bleached. Underlying blades (the blades overlap one another) may survive.

Transport and survival in diluted sea water. A 6–8-hour exposure to rain could result in serious injury or death, as indicated by our experiments. Lack of Ca results in rapid loss of K and of weight, and these losses are speeded during osmotic stress. In 100 per cent sea water, free of Ca, *Porphyra* may survive up to 30 hours, compared with only 6–8 hours in 30 per cent Ca-free sea water.

Lack of potassium (Eppley, 1958b) also may influence survival because of the necessity of K for extrusion of Na. Unpublished results indicate that respiration is inhibited about 60 per cent in the absence of Na. Thus the presence of Ca, K, and Na (and probably also Mg) is required for normal operation of cellular processes, one of which is ion transport.

It is of some interest that even in distilled water *Porphyra* tissues adsorb about 10 mM/Kg. FW of Ca. This amount, probably associated with the extracellular galactan sulfate, is not apparently available to the critical sites involved in maintaining membrane selectivity. The implication of this finding may be of some importance in clarifying the role of trace elements passively adsorbed to extracellular polysaccharides in algae. Thus trace element "accumulation" (Black and Mitchell, 1952), in which the extent of adsorption was not determined, might be re-interpreted in the light of this result, with respect to the survival value of such "accumulation." The likelihood remains, however, that in the presence of exchangeable cations, adsorbed trace elements could be made available to the cell surfaces for real accumulation.

Growth experiments which might clarify this problem have not been possible, due to our failure to obtain growth of *Porphyra* in the laboratory. The recent results of Kanazawa and Kashiwada (1959) on the culture of *Porphyra tenera* cast some hope on our aspirations of doing so, however.

A heavy rain, by washing away the sea water film normally present between and around the blades even during emergence, would almost certainly abolish membrane selectivity, decrease respiration, induce loss of cellular cations, and result in high mortality if the blades were exposed long enough. In southern California *Porphyra perforata* largely disappears in the winter, and rain may be involved in this mortality. Other factors are also involved, however.

At the site studied, approximately two miles north of the Los Angeles-Ventura County boundary in California, mass movements of sand were observed during the winter of 1958–1959. The old *Porphyra* bed was entirely covered and a new bed appeared about 50 yards south in the following spring. A smaller sand movement partially covered the new bed in July, 1959. While the extracellular polysaccharides may act as cushions against moderate wave impact and sand scouring, the plants may be torn loose or shredded in a heavy surf or be buried by sand

movements. Such physical processes may be important in survival and distribution as well as physiological tolerances.

Summary

1. Over a wide range of salinity (10 to 200 per cent sea water) cells of *Porphyra perforata* accumulate K and partially exclude Na. Apparent osmotic volume is nearly constant between 50 and 100 per cent sea water. This imperfect volume control is thought to be due to differential shrinking and swelling of structural polysaccharides and not to active water secretion.

2. Survival of *Porphyra* in diluted and concentrated sea water is discussed with respect to ion transport. Rain is potentially a more serious threat to survival, due in part to breakdown of ion transport, than is concentration of sea water. Calcium seems especially important in maintaining membrane selectivity toward K and Na.

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